

## SEQUENCE LISTING

<110> NAGAI, Hiroshi  
NAKAJIMA, Terumi

<120> NOVEL HEMOLYTIC ACTIVE PROTEINS AND GENES ENCODING THE  
SAME

<130> PCTJP9901607NAGAIHiroshietal 183049264

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<150> PCT/JP99/01607

<151> 1999-03-30

<150> JP 10/88569

<151> 1998-04-01

<160> 18

<170> PatentIn Ver. 2.0

<210> 1

<211> 14

<212> PRT

<213> Carybdea rastonii

<220>

<223> This amino acid residue sequence corresponds to  
amino acid residue positions 56-69 of SEQ ID NO:5.

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Gly Glu Ile Gln Thr Lys Pro Asp Arg Val Gly Gln Ala Thr

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<210> 2

<211> 18

<212> PRT

<213> Carybdea rastonii

<220>

<223> This amino acid residue sequence corresponds to  
amino acid residue positions 250-267 of SEQ ID  
NO:5.

<400> 2

Gly Asn Ala Glu His Val Ala Ser Ala Val Glu Asn Ala Asn Arg Val  
 1 5 10 15

Asn Lys

<210> 3  
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 <213> Carybdea rastonii

<220>  
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 Met Ile Leu Lys His Leu Pro Trp Leu  
 1 5

ttt att gtc ctt gca att act tct gca aaa cat ggc aaa cgc tct gat 102  
 Phe Ile Val Leu Ala Ile Thr Ser Ala Lys His Gly Lys Arg Ser Asp  
 10 15 20 25

gtc aat tct tta ctt act aag gta gaa act gcc tta aaa gaa gct tct 150  
 Val Asn Ser Leu Leu Thr Lys Val Glu Thr Ala Leu Lys Glu Ala Ser  
 30 35 40

26

ggt agc aac gag gct gct ctt gag gct tta gag ggc tta aaa gga gag	198
Gly Ser Asn Glu Ala Ala Leu Glu Ala Leu Glu Gly Leu Lys Gly Glu	
45 50 55	
atc cag aca aaa cca gac cga gtt gga caa gcc aca aaa atc ctt gga	246
Ile Gln Thr Lys Pro Asp Arg Val Gly Gln Ala Thr Lys Ile Leu Gly	
60 65 70	
tct gtc gga tca gct cta gga aaa tta aat tct gga gat gca acc aaa	294
Ser Val Gly Ser Ala Leu Gly Lys Leu Asn Ser Gly Asp Ala Thr Lys	
75 80 85	
atc att tct ggt tgc ctc gac att gtt gca gga att gca aca act ttt	342
Ile Ile Ser Gly Cys Leu Asp Ile Val Ala Gly Ile Ala Thr Thr Phe	
90 95 100 105	
gga ggc cct gtc ggg atg gga atc gga gcc gta gct tct ttt gtt tct	390
Gly Gly Pro Val Gly Met Gly Ile Gly Ala Val Ala Ser Phe Val Ser	
110 115 120	
tca att cta tca ttg ttt act gga agc tca gca aag aac tca gtt gct	438
Ser Ile Leu Ser Leu Phe Thr Gly Ser Ser Ala Lys Asn Ser Val Ala	
125 130 135	
gcc gtt att gat aga gct tta agc aag cat cgc gat gag gcc atc caa	486
Ala Val Ile Asp Arg Ala Leu Ser Lys His Arg Asp Glu Ala Ile Gln	
140 145 150	
aga cat gca gca ggt gcc aag aga gat ttt gct gaa tca tct gca ttc	534
Arg His Ala Ala Gly Ala Lys Arg Asp Phe Ala Glu Ser Ser Ala Phe	
155 160 165	
att cag gtc atg aaa cag cag tcc aat ctt aca gat agc gac cta agt	582
Ile Gln Val Met Lys Gln Gln Ser Asn Leu Thr Asp Ser Asp Leu Ser	
170 175 180 185	
atc att gca gcg aat gtt cct gtt tat aaa ttt agt aat ttt atc gga	630
Ile Ile Ala Ala Asn Val Pro Val Tyr Lys Phe Ser Asn Phe Ile Gly	
190 195 200	
cag ttg gag agc aga att tcc caa ggc gca gca act acc agt ctt agc	678
Gln Leu Glu Ser Arg Ile Ser Gln Gly Ala Ala Thr Thr Ser Leu Ser	
205 210 215	
gat gca aag aga gcc gtt gac ttc att ctg ctc tat tgt caa ctt gta	726
Asp Ala Lys Arg Ala Val Asp Phe Ile Leu Leu Tyr Cys Gln Leu Val	
220 225 230	

gtc atg aga gaa acc ttg ctg gtc gac ttg gct att ctc tac agg aaa	774
Val Met Arg Glu Thr Leu Leu Val Asp Leu Ala Ile Leu Tyr Arg Lys	
235 240 245	
gga aat gca gaa cac gtg gca agt gct gtg gaa aac gct aat agg gta	822
Gly Asn Ala Glu His Val Ala Ser Ala Val Glu Asn Ala Asn Arg Val	
250 255 260 265	
aac aaa gag cta gct gct gat acc cta gat ttt ctt cat aaa ttg att	870
Asn Lys Glu Leu Ala Ala Asp Thr Leu Asp Phe Leu His Lys Leu Ile	
270 275 280	
cct gaa caa gca ttg ata ggt gca gtt tat cat cca att tct gcc tct	918
Pro Glu Gln Ala Leu Ile Gly Ala Val Tyr His Pro Ile Ser Ala Ser	
285 290 295	
gaa act agc aaa gca ata tta aat tac acg aaa tac ttt gga gtt cca	966
Glu Thr Ser Lys Ala Ile Leu Asn Tyr Thr Lys Tyr Phe Gly Val Pro	
300 305 310	
gat gtt ccc cgt cct att gga aac cgc aga tac aaa ttt aca aat agt	1014
Asp Val Pro Arg Pro Ile Gly Asn Arg Arg Tyr Lys Phe Thr Asn Ser	
315 320 325	
tac tgg aat acc tac agt ata tgc agt gag gct tac atg gga aat tac	1062
Tyr Trp Asn Thr Tyr Ser Ile Cys Ser Glu Ala Tyr Met Gly Asn Tyr	
330 335 340 345	
atg ttc aga ggc tgt tct aac gtt cgg aat cca aat atc agg gta tcc	1110
Met Phe Arg Gly Cys Ser Asn Val Arg Asn Pro Asn Ile Arg Val Ser	
350 355 360	
aaa atg tct gat ggg ttt tac acc atg gag aat agc gat cgg agg aag	1158
Lys Met Ser Asp Gly Phe Tyr Thr Met Glu Asn Ser Asp Arg Arg Lys	
365 370 375	
ttg tat atc acc aag cat gac caa gga tgg gga tgg ggt act ttg gat	1206
Leu Tyr Ile Thr Lys His Asp Gln Gly Trp Gly Trp Gly Thr Leu Asp	
380 385 390	
gag gat cca ggt gac caa ggc cat atg agg ttc att cct ttg aga cat	1254
Glu Asp Pro Gly Asp Gln Gly His Met Arg Phe Ile Pro Leu Arg His	
395 400 405	
ggg aag tat atg gta agc tct aag agg tgg ccc aac tgg ttc atg tat	1302
Gly Lys Tyr Met Val Ser Ser Lys Arg Trp Pro Asn Trp Phe Met Tyr	
410 415 420 425	

atg gaa tca agt gcc agt ggc tac att cgc agc tgg gaa aat aat cca 1350  
 Met Glu Ser Ser Ala Ser Gly Tyr Ile Arg Ser Trp Glu Asn Asn Pro  
 430 435 440

gga cct caa gga cat tgg agt ata aca taa ttaaagagga atcaacaatg 1400  
 Gly Pro Gln Gly His Trp Ser Ile Thr  
 445 450

tcccaaaggc atacgaatat aagacatcaa acgaatgcag tacttaaagt gcacacttgt 1460  
 atttctacat aggatgtcgt catgaaagtc cataaaccat ccagcggact aatttcatat 1520  
 taaacattaa tgtttcctta taatgcattt tcatgaaatc tctattgtga catttcaaga 1580  
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 <212> PRT  
 <213> Carybdea rastonii

<400> 5  
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Ser Ala Lys His Gly Lys Arg Ser Asp Val Asn Ser Leu Leu Thr Lys  
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Val Glu Thr Ala Leu Lys Glu Ala Ser Gly Ser Asn Glu Ala Ala Leu  
 35 40 45

Glu Ala Leu Glu Gly Leu Lys Gly Glu Ile Gln Thr Lys Pro Asp Arg  
 50 55 60

Val Gly Gln Ala Thr Lys Ile Leu Gly Ser Val Gly Ser Ala Leu Gly  
 65 70 75 80

Lys Leu Asn Ser Gly Asp Ala Thr Lys Ile Ile Ser Gly Cys Leu Asp  
 85 90 95

Ile Val Ala Gly Ile Ala Thr Thr Phe Gly Gly Pro Val Gly Met Gly  
 100 105 110

Ile Gly Ala Val Ala Ser Phe Val Ser Ser Ile Leu Ser Leu Phe Thr  
 115 120 125

Gly Ser Ser Ala Lys Asn Ser Val Ala Ala Val Ile Asp Arg Ala Leu

130	135	140
Ser Lys His Arg Asp Glu Ala Ile Gln Arg His Ala Ala Gly Ala Lys		
145	150	155 160
Arg Asp Phe Ala Glu Ser Ser Ala Phe Ile Gln Val Met Lys Gln Gln		
165	170	175
Ser Asn Leu Thr Asp Ser Asp Leu Ser Ile Ile Ala Ala Asn Val Pro		
180	185	190
Val Tyr Lys Phe Ser Asn Phe Ile Gly Gln Leu Glu Ser Arg Ile Ser		
195	200	205
Gln Gly Ala Ala Thr Thr Ser Leu Ser Asp Ala Lys Arg Ala Val Asp		
210	215	220
Phe Ile Leu Leu Tyr Cys Gln Leu Val Val Met Arg Glu Thr Leu Leu		
225	230	235 240
Val Asp Leu Ala Ile Leu Tyr Arg Lys Gly Asn Ala Glu His Val Ala		
245	250	255
Ser Ala Val Glu Asn Ala Asn Arg Val Asn Lys Glu Leu Ala Ala Asp		
260	265	270
Thr Leu Asp Phe Leu His Lys Leu Ile Pro Glu Gln Ala Leu Ile Gly		
275	280	285
Ala Val Tyr His Pro Ile Ser Ala Ser Glu Thr Ser Lys Ala Ile Leu		
290	295	300
Asn Tyr Thr Lys Tyr Phe Gly Val Pro Asp Val Pro Arg Pro Ile Gly		
305	310	315 320
Asn Arg Arg Tyr Lys Phe Thr Asn Ser Tyr Trp Asn Thr Tyr Ser Ile		
325	330	335
Cys Ser Glu Ala Tyr Met Gly Asn Tyr Met Phe Arg Gly Cys Ser Asn		
340	345	350
Val Arg Asn Pro Asn Ile Arg Val Ser Lys Met Ser Asp Gly Phe Tyr		
355	360	365
Thr Met Glu Asn Ser Asp Arg Arg Lys Leu Tyr Ile Thr Lys His Asp		
370	375	380
Gln Gly Trp Gly Trp Gly Thr Leu Asp Glu Asp Pro Gly Asp Gln Gly		

385                      390                      395                      400  
 His Met Arg Phe Ile Pro Leu Arg His Gly Lys Tyr Met Val Ser Ser  
                          405                      410                      415  
 Lys Arg Trp Pro Asn Trp Phe Met Tyr Met Glu Ser Ser Ala Ser Gly  
                          420                      425                      430  
 Tyr Ile Arg Ser Trp Glu Asn Asn Pro Gly Pro Gln Gly His Trp Ser  
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<210> 6  
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<220>  
 <223> Description of Artificial Sequence: Degenerate PCR  
       primer, 7-F, used in the cloning of the partial  
       cDNA of the hemolytic active protein of *Carybdea*  
       *rastonii*

<220>  
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 <222> (12)  
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<220>  
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19

<210> 7  
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19

<210> 8

<211> 20

<212> DNA

<213> Artificial Sequence

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<222> (15)

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<210> 9



<211> 20  
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<223> Description of Artificial Sequence: Degenerate PCR  
primer, 12-R, used in the cloning of the partial  
cDNA of the hemolytic active protein of *Carybdea*  
*rastonii*

<400> 9  
ckrttngcrt tytcnacngc

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<210> 10  
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cDNA of the hemolytic active protein of *Carybdea*

rastonii

<400> 10

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<210> 11

<211> 19

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<223> Description of Artificial Sequence: Degenerate PCR  
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cDNA of the hemolytic active protein of Carybdea  
rastonii

<400> 11

ccatngtrta raancrrtc

19

<210> 12

<211> 23

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*rastonii*

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<210> 13

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<220>

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<223> n = inosine

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*rastonii*

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ttytccatng trtaraancc rtc

23

<210> 14

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: 5' Race  
primer, 5'-RACE-4R, synthesized based on the base  
sequence of the partial cDNA for the hemolytic  
active protein of *Carybdea rastonii*

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<210> 15  
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<210> 16  
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<223> Description of Artificial Sequence: 5' Race  
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<210> 17  
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<213> Artificial Sequence

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<223> Description of Artificial Sequence: 3' Race  
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sequence of the partial cDNA for the hemolytic  
active protein of *Carybdea rastonii*

<400> 17  
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<210> 18  
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<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: 3' RACE  
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sequence of the partial cDNA for the hemolytic  
active protein of *Carybdea rastonii*

<400> 18

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20